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Detection of Enteropathogens in Fatal and Potentially Fatal Diarrhea in Cairo, Egypt

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A 1-year study of the etiology of acute diarrhea complicated by severe (10%) dehydration, active bleeding, shock and cardiovascular collapse, pneumonia, acute renal failure, or seizures in infants under 18 months of age was performed in Cairo, Egypt. Of 145 infants, 19 (13%) died or left the hospital moribund; the remaining 126 patients were classified as having potentially fatal illness. A variety of enteropathogens were identified with approximately equal frequency in the fatal and nonfatal complicated cases as well as in 135 controls with severe uncomplicated diarrhea. The agents most frequently detected in infants with severe diarrhea in this population which were felt to be etiologically important were rotavirus (33%), heat-stable enterotoxin-producing *Escherichia coli* (20%), heat-labile enterotoxin-producing *E. coli* (11%), enteropathogenic *E. coli* (8%), and *Salmonella* spp. (5%). The high rate of occurrence of *Giardia lamblia* (35%) probably represented the high carriage rate of the protozoan in this population. Complicated (fatal and potentially fatal) cases differed from control cases in a number of ways: the onset of diarrhea was more sudden, the course was progressive and of greater initial intensity, vomiting occurred more frequently, the patients more often had visited another physician before coming to the hospital, the patients more often had respiratory symptoms and pulmonary abnormalities on auscultation, hypoactive bowel sounds and abdominal distention were more common, as was oliguria, and the patients showed lower mean body weights.

Gastroenteritis remains an important cause of morbidity in all areas of the world. In many regions of the developing world, diarrhea also represents a leading cause of infant mortality. It has been estimated that 4.6 million deaths occur annually among children under 5 years of age in Africa, Asia (excluding China), and Latin America (17). In Egypt, as reported to the Ministry of Health, diarrhea and malnutrition account for 54% of total infant mortality (11). Death from diarrhea is most common in infants under 1 year of age.

Since the mid-1970s, techniques have been available to detect rotavirus, enterotoxigenic *Escherichia coli* (ETEC), and *Campylobacter* spp. Collectively, these agents account for 20 to 50% of diarrhea in tropical areas (1, 2, 9, 10). However, little information is available concerning the etiologic agents associated with fatal diarrhea. This is at least partially explained by the fact that mortality can be prevented when a population is placed under study, due to the beneficial effect of rehydration. This study was designed to characterize the agents associated with fatal and potentially fatal complicated diarrhea occurring in Egypt's largest pediatric inpatient facility. This medical center was uniquely suited for this study, because it is known that infants commonly are admitted with severe and complicated diarrheal illness, a percentage of which fails to respond to appropriate fluid therapy.

MATERIALS AND METHODS

The study was performed at a university pediatric hospital in Cairo during a 12-month period, May 1982 through April 1983. The institution serves Cairo and the surrounding suburban and rural areas. Most patients are of low-to-middle socioeconomic status. Children brought to this hospital for

evaluation and therapy are usually severely ill. Children with complicated illness are admitted to the hospital for treatment and clinical monitoring, and children with uncomplicated illness are kept in a rehydration room until properly hydrated, at which time they are discharged. Infants ≤18 months of age seen in the outpatient clinic with the complaint of diarrhea and with potentially fatal complications of their illness were eligible for enrollment in the study. To be included, an infant must have had diarrhea of no more than 4 days' duration. Complications of diarrhea included severe (≥10%) dehydration, active bleeding or hemorrhage, acidosis (serum pH <7.3), oliguria (<400 ml of urine per m² per day), and seizures. Each enrolled patient with complicated illness was matched with an infant under 18 months of age coming to the same clinic the same day with similar but uncomplicated diarrhea, who served as a control. While priority for enrollment was given to patients with a negative history of antidiarrheal or antimicrobial therapy, 32% of the patients with particularly severe complicated illness were enrolled after having received one or two doses of preadmission therapy. Controls were excluded if they had received antimicrobial therapy. Each infant studied was required to furnish an illness stool specimen, which was processed for enteropathogens.

At the time of enrollment in the study, a history, which included clinical and epidemiologic data, was taken from the family spokesperson (usually the mother). A physical examination was performed. All patients with complicated illness were admitted to the hospital. Additionally, pH, osmolality, urea nitrogen, and serum electrolytes were ascertained from a blood sample by manual techniques. The subjects were treated with fluids and electrolytes, either orally (4) or intravenously. Standard therapy consisted of oral therapy (ORS) recommended by the World Health Organization (4). Intravenous fluids were restricted to use in cases of intense

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diarrhea and persistent vomiting with 10% dehydration or shock.

All illness stools were classified as to form and examined for *Giardia lamblia*, *Entamoeba histolytica*, shigellae, salmonellae, campylobacters, yersiniae, and heat-labile enterotoxin (LT)-producing and serotype-identified *E. coli*. Salmonellae and shigellae were identified biochemically after stool samples were plated directly and incubated overnight in Selenite Broth onto MacConkey (Difco Laboratories, Detroit, Mich.), salmonella-shigella (Difco), and Xylose-Lysine Dextrose (Difco) agars. *Campylobacter jejuni* was sought by plating stools onto modified Skirrow's agar and identified by growth characteristics and Gram stain (14). *Yersinia enterocolitica* was isolated from MacConkey agar after a 3-week enrichment in phosphate-buffered saline. For enteropathogenic *E. coli* (EPEC) serotyping, five colonies from each stool specimen to be studied were tested by slide agglutination against commercially available (BioMérieux, Charbonnièrs-les-Bains, France) antisera. Within 1 month of isolation, approximately five *E. coli* colonies per specimen were assayed individually in Y-1 adrenal cells (7) for the presence of choleralike *E. coli* LT. Half of the specimens selected at random were assayed for the presence of heat-stable enterotoxin (ST)-producing *E. coli* in the suckling mouse model (6) after filtrates from the five *E. coli* colonies were pooled (3). *E. coli* producing either LT or ST was considered to be ETEC. Selected colonies of *E. coli* identified in Cairo as EPEC or ETEC had their identification verified in Houston at The University of Texas enteric disease research laboratory. Parasites were identified in Cairo by microscopic examination of a Wheatley's trichrome-stained fecal smear and after treatment with merthiolate-iron-Formalin (18). Rotavirus was identified by a modification of the enzyme-linked immunosorbent assay, which included a confirmation of selected positive and negative samples at the National Institutes of Health (12).

Statistical comparisons were made by chi-square analysis, Student's t test, and analysis of variance.

RESULTS

During the 12-month study, 145 infants with various complications of diarrheal illness were enrolled. The complications occurring in these infants were severe ($\geq$10%) dehydration in 123 (85%) patients, active bleeding in 12 (8%) patients, shock and cardiovascular collapse in 4 (3%) patients, pneumonia in 4 (3%) patients, and seizures and acute renal failure in 1 patient each (1%). Nineteen of the patients with complicated diarrhea (13%) either died or were taken in a moribund state from the hospital by a parent and in the judgment of the attending physician were not expected to recover from their illness. These patients were considered to have fatal illness. The control population consisted of 135 infants with uncomplicated diarrhea. Of the 280 infants studied, 54% were males and 46% were females; 25% lived in rural areas of Egypt, and 75% were from urban Cairo. The mean ages and age ranges for the three groups were as follows: for infants with fatal diarrhea, 217 and 65 to 387 days; for infants with nonfatal complicated diarrhea, 230 and 46 to 526 days; and for infants with uncomplicated diarrhea, 227 and 17 to 519 days.

The frequencies of the specific enteropathogens identified in the two groups were similar (Table 1). The enteropathogens found most commonly in the study which were believed to have etiologic importance were rotavirus (33%), ST ETEC (20%), LT ETEC (11%), EPEC (8%), and *Salmo-*

TABLE 1. Enteropathogens isolated from infants with severe diarrhea according to the presence or absence of complications in Cairo from 1982 to 1983

Agent sought	Patients with complicated diarrhea		Patients with uncomplicated diarrhea		All patients	
	No. tested	No. (%) positive	No. tested	No. (%) positive	No. tested	No. (%) positive
Rotavirus	137	47 (34)	131	41 (31)	268	88 (33)
Shigella	142	1 (1)	133	1 (1)	275	2 (1)
Salmonella	142	11 (8)	133	4 (3)	275	15 (5)
Campylobacter	145	1 (1)	135	4 (3)	280	5 (2)
EPEC[a]	137	13[b] (9)	129	8 (6)	266	21 (8)
LT ETEC	137	12[b] (9)	129	18 (14)	266	30 (11)
ST ETEC	49	9 (18)	77	16 (21)	126	25 (20)
E. histolytica	118	0	118	0	236	0
None[c]	40	9 (23)	66	17 (26)	106	26 (25)

[a] Determined by serotype.
[b] One patient had both EPEC and LT ETEC in the stool.
[c] Results given for specimens examined for all agents.

nella spp. (5%). *G. lamblia* was identified in 35% of the cases (data not shown). Of 106 cases in which all agents were sought, 26 (25%) were unassociated with a detectable pathogen in the stool. The agents identified in the 19 fatal cases occurred with similar frequency when compared with the other two groups, although only 2 of 19 patients (11%) were found to be positive for rotavirus. The other agents identified were EPEC in 3 of 18 patients (17%), LT ETEC in 2 of 18 patients (11%), ST ETEC in 1 of 5 patients (20%), and *G. lamblia* in 5 of 13 patients (38%). One of the infants with fatal illness had both an EPEC and an LT ETEC in the diarrheal stool studied.

For comparative purposes, three groups are specified: fatal diarrhea (19 infants), nonfatal complicated diarrhea (126 infants), and uncomplicated diarrhea (135 infants). Historically, the fatal and nonfatal complicated groups looked similar, and both by analysis of variance were significantly ($P < 0.004$) different from the uncomplicated group in a number of ways (Table 2). The patients with complicated diarrhea (including fatal diarrhea) characteristically had histories of diarrhea of sudden onset, greater initial (first 24 h) intensity of diarrhea ($\geq$ seven unformed stools), a course classified as progressive, more often being taken to another physician before being brought to the hospital, more frequent vomiting, more often having had fever, and respiratory symptoms. Differences in physical findings were also noted. More of the patients with complicated diarrhea were clinically less alert, which was particularly so for the patients with fatal illness. Also, patients with complicated diarrhea more often had hypoactive bowel sounds and abdominal distention, pulmonary abnormalities on auscultation (rhonchi, rales, and wheezes), and oliguria. Finally, the mean weight (but not length) of the infants with complicated disease was lower: infants with fatal diarrhea, 6.09 kg; infants with nonfatal complicated diarrhea, 6.19 kg; and infants with uncomplicated diarrhea, 6.7 kg ($P = 0.03$). The respiratory rate ($P < 0.0001$) and pulse ($P < 0.0001$) were increased in the patients with complicated illness relative to the group with uncomplicated illness.

Laboratory studies were done only for the complicated cases. Admission blood pH averaged 7.14 $\pm$ 0.39 for the 19 patients with fatal disease compared with 7.23 $\pm$ 0.09 for 101 of the patients with nonfatal complicated disease ($P = 0.0005$). The average hematocrit was higher in the patients with fatal diarrhea than in patients with nonfatal complicated

TABLE 2. Historical aspects and physical findings in hospitalized infants with diarrhea in Cairo from 1982 to 1983[a]

Historical or clinical finding	No. (%) in patient group		
	Fatal diarrhea	Nonfatal complicated diarrhea	Uncomplicated diarrhea
Sudden onset	16 (84)	82 (65)	54 (40)
≥7 Unformed stools, first 24 h	18 (95)	112 (89)	66 (49)
Progressive course	13 (68)	86 (68)	29 (21)
Visit to another physician	7 (37)	48 (38)	27 (20)
Vomiting			
Persistent	7 (39)	60 (49)	31 (24)
Occasional	10 (56)	51 (42)	52 (40)
None	1 (6)	11 (9)	48 (37)
History of fever	18 (95)	110 (87)	66 (49)
Respiratory symptoms	14 (74)	93 (74)	45 (33)
Alertness	9 (47)	97 (77)	133 (99)
Dehydration (≥10%)	19 (100)	104 (83)	0
Bleeding	8 (42)	4/125[b] (3)	0
Hypoactive bowel sounds	6 (32)	20/126[b] (16)	2 (1)
Abdominal distention	9 (47)	26/126[b] (21)	3 (2)
Abnormal pulmonary auscultation	16 (84)	84/126[b] (67)	18 (13)
Oliguria	14/17[b] (82)	86/115[b] (75)	11/132[b] (8)

[a] Findings were significantly different ($P < 0.004$) by analysis of variance.
[b] Not all patients were evaluated.

diarrhea ($P = 0.03$). The remaining laboratory values were similar in the two groups. Direct causes of death of patients with fatal disease included pneumonia, sepsis, severe metabolic acidosis, and disseminated intravascular coagulation. Severe uncomplicated dehydration was not believed to have been a cause of death. The stools passed by children in all three groups were similar in terms of form (watery, mixed, or soft) and presence of blood, mucus, or leukocytes. Similar frequencies were seen in the three groups for sex and rural versus urban location of homes, episodes of diarrhea in other family members during the 2 weeks before admission of the patient to the study, frequency of diarrhea in the patient under study over the past year or time since the last episode, education of mother, occupation of father, number of siblings in the household, number of rooms in the house, presence of animals in the house, source of drinking water, and whether the infant was breast fed, was given boiled water, or had been started on milk other than breast milk or received other foods.

DISCUSSION

Diarrheal morbidity rates do not show striking differences when developing or tropical areas are compared with highly industrialized regions, but mortality rates are very different (15). Little information is available concerning the cause of the approximately 4.6 million infant deaths which occur in the developing world (17), despite the availability of techniques to identify the etiology of most cases of acute diarrhea studied by research laboratories. This study was designed to determine the cause of fatal and potentially fatal diarrheal illness occurring in the largest pediatric center in Egypt.

The agents associated with fatal and potentially fatal diarrhea were similar in frequency to those of a control group of infants brought to the same hospital with severe

diarrhea without complications. Rotavirus, ETEC (ST and LT producing), EPEC, and Salmonella spp. were identified in 66% of the cases studied. The rates were considerably higher than those we observed in a rural Egyptian outpatient population studied simultaneously by the same laboratory, for which the same four agents were found in 32% of cases of diarrhea in infants under 1 year of age (20). The most striking difference was seen for rotavirus, which was implicated in 34% of the complicated illness in the present study but found by us in only 5% of outpatient diarrhea occurring in infants under 1 year of age living in rural Bilbeis, Egypt (20). Diarrheal illness seen in patients coming to the hospital is likely to be more severe and probably is more likely to be associated with stool pathogens than illness occurring in persons studied prospectively in an ambulatory setting. This implies that much of the undiagnosable milder disease seen in an ambulatory setting results from noninfectious causes or agents not yet well described. EPEC serotypes were identified in 8% of the infants brought to the hospital with severe diarrhea in the present study. Although the pathogenicity of the EPEC strains has not been entirely clarified, growing evidence suggests they are important causes of human illness (8). EPEC strains often show a characteristic virulence property, mannose-resistant adherence to HEp-2 cells (5), and illness has been produced in adult volunteers after experimental challenge (13). The high rate of G. lamblia organisms identified in the present study (35%) was actually lower than the frequency with which we found the agent in asymptomatic controls in the rural Egyptian population (56%). Others have shown that in Bangladesh rotavirus and ETEC (along with Vibrio cholerae) are the major causes of dehydration (1, 2). The patients with potentially fatal diarrhea in the present study characteristically had histories of diarrhea of more sudden onset, passage of a greater number of stools during the first 24 h of illness, and increased vomiting when compared with control patients with uncomplicated diarrhea, factors that undoubtedly contributed to severe dehydration.

Although many of the agents identified in this study appeared capable of producing serious illness requiring hospitalization for evaluation and therapy, only rotavirus occurred with obviously greater frequency when this severe hospital-based diarrhea was compared with milder outpatient illness as studied simultaneously by our group (20). It appeared in the present study that host or therapeutic factors rather than microbial factors were more important to the development of a potentially fatal complication once patients developed severe illness. We draw this conclusion because the agents appeared with equal frequency in patients with severe diarrhea with or without potentially fatal complications. Host factors requiring further study include state of nutrition, presence of hypochlorhydria or abnormal patterns of gastric emptying, deficiency of intestinal immunoglobulins or cell-mediated immune mechanisms, and presence of abnormal intestinal flora or their metabolites. Obvious therapeutic factors which relate to recovery in clinically severe diarrheal disease are orally and parenterally administered fluids and electrolytes and, in selected cases, antimicrobial agents. The findings of the present study, in which multiple enteropathogens were encountered, support the use of nonspecific forms of therapy in attempting to minimize mortality, such as oral rehydration (16). In that regard, effective educational programs and rehydration centers probably will be required to decrease diarrhea-related morbidity or mortality in the developing world because of a lack of general education and awareness of prevention and therapeutic

principles in these areas (4). That approximately one-third of the infants with severe diarrhea were found to be infected by rotaviruses also supports efforts toward developing and evaluating rotavirus vaccines (19).

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